THE RELATIONSHIP OF PROTEIN DEFICIENCY TO SURGICAL INFECTION*

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THE CONSEQUENCES of a prolonged period of negative nitrogen balance in a surgical patient will depend upon the preoperative abundance of the protein reserves and the postoperative degree of their depletion.[1, 2, 3, 4, 5, 6] The significance of these reserves is even yet not fully realized despite the many recent studies indicating their dynamic relationship to the plasma proteins.[7, 8] Their amount and availability, however, constitute an important safety-factor in that, as a rule, a postoperative loss of nitrogen will be less serious, coming from abundant protein stores than from ones already depleted because of protracted debilitating disease. Moreover, if before operation upon a hypoproteinemic patient, the tissue-protein deficit can be at least partially corrected by the administration of high-quality proteins, the chances of survival should be considerably improved, provided that the mechanism responsible for blood protein synthesis has not been too severely damaged by the underlying pathologic process.

Nutritional emphasis upon protein metabolism in surgical conditions has usually centered around such problems as the relationship of albumin loss, regeneration and replacement, to surgical shock, burns, blood loss, nutritional edema, wound healing, liver impairment and anesthetic injury, and comparatively little consideration has been given to the function of proteins in the prevention or amelioration of postoperative infection. Yet infection is an ever-present surgical menace, and the best surgical technic may be of slight avail in a markedly hypoproteinemic patient. Although infection may develop even under conditions of good nutrition, it is more likely to become menacing or even lethal when basic immunologic mechanisms are seriously impaired. For example, infection tends to become especially threatening in association with marked malnutrition, starvation or wasting disease, or with such chronic debilitating diseases as malignancy (especially of the gastro-intestinal tract), nephritis, cirrhosis of the liver, ulcerative colitis and the like, where inanition is actually a prominent part of the pathologic picture.[9, 10]

Nevertheless, such patients may be operated upon with little realization by the surgeon that they are in the danger zone of severe hypoproteinemia.

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The following brief case history illustrates the latter point. A white female, 35 years of age, was operated upon for the removal of a carcinoma of the rectum. The operation, a combined abdomino-perineal resection, was performed under continuous spinal anesthesia. For 24 hours postoperatively the patient did well; then the temperature rose to 103.6° F. The posterior incision was opened and 5 Gm. of sulfanilamide were placed in the wound, but despite this and various supportive measures she died on the third postoperative day. Necropsy revealed a generalized fibrinopurulent peritonitis. Immediately after death cardiac blood was taken for total protein estimation; this was 3.98 Gm. per 100 Ml. of serum. The history revealed that during the last two years she had lost 52 pounds in weight (159 pounds to 107 pounds). This severe loss of weight reemphasizes the fact stressed by Studley[11] that postoperative fatality rates tend to parallel the extent of the preoperative weight loss. Thus, Studley observed, in patients who had had a preoperative weight loss of more than 20 per cent of their normal weight, a postoperative fatality rate of over 30 per cent. In several of these patients, moreover, there was wound disruption and terminal pneumonia.

Pneumonia and other types of postoperative infection obviously develop because the primary defense barriers have failed to prevent bacterial penetration into the deeper tissues; the consequences of this failure must depend upon the combined and complementary activities of the forces of natural and acquired resistance and the ability of these forces to restrict further microbic growth and spread. An important question is, therefore: Do nutritional conditions influence these two types of resistance, and, if so, how?

It is to be expected that profound undernutrition and its concomitant depletion of the protein reserves should influence adversely the mechanisms of *natural* resistance, because a protracted period of protein deficiency leads eventually to marked atrophy of the liver, spleen, bone marrow and lymphoid tissues; and from these tissues most of the phagocytic cells originate. Moreover, inasmuch as protein is the basic material from which all tissues ultimately are constructed, the absence of protein stores necessary for further construction of leukocytic reserves might also reduce the continued production of phagocytes, particularly if protein synthesis were further impaired as a consequence of an advancing infection.

Of equal, if not greater, importance with respect to the harmful potentiality of many of the common pathogenic micro-organisms is *acquired* resistance. Acquired resistance differs from natural resistance mainly because of the antibody mechanism, and there is no reason to believe that antibody action is restricted to only particular kinds of infection. In fact, all pathogenic bacteria, once they have entered the tissues, are foreign proteins; hence they are antigenic stimulants, both locally and generally, of the antibody mechanism. Antibodies, collaborating with phagocytes, promote bacterial localization and thus help to bring incipient infections quickly to an end. It is only when antibodies are weakly formed, as, for example, when the antibody mechanism functions at a lowered level, that anti-

bacterial defense presumably must depend upon the latent potentialities of natural resistance. It would seem obvious, therefore, that the capacity of the antibody mechanism to respond effectively will determine both the extent and outcome of many types of infection, especially those which are otherwise imperfectly combatted by the forces of natural resistance.

In an effort to ascertain more precisely the nature and functions of the antibody mechanism we have been investigating its relationship to protein metabolism. The rôle of proteins in antibody production did not become apparent until after the demonstration that an antibody is actually a modified tissue protein, a molecule of globulin. This fact suggests, however, that the problem of antibody production is but a part of the larger problem of protein synthesis. In a series of publications we have presented evidence supporting this hypothesis and indicating that both the acquisition and retention of specific resistance may be determined largely by an adequate intake and utilization of dietary protein.[12, 13, 14]

In the present paper additional evidence is submitted demonstrating that impairment of the antibody mechanism is one of the sequelae of protein undernutrition and that, when there is a lack of essential amino-acids normally required for the formation of serum globulins, antibody synthesis cannot proceed optimally, even if all the other known necessary dietary factors are present. Before presenting this evidence, however, a few of the reasons supporting our general hypothesis will be summarized briefly under three categories, *viz.*, (1) the relationship of protein ingestion to the synthesis of normal globulin; (2) the relationship of protein ingestion to the synthesis of antibody globulin; and, (3) the relationship of protein deficiency to antibody synthesis and antibacterial resistance.

I. THE RELATIONSHIP OF PROTEIN INGESTION TO THE SYNTHESIS OF NORMAL GLOBULIN

There is now good evidence that the fabrication of normal serum globulin is dependent upon the utilization of an adequate assortment of amino acids. For example, Whipple, and his associates, have shown "that globulin formation is directly dependent upon diet." Thus, in summarizing their experimental findings Madden and Whipple say[8] "the same 100 grams of beef serum which produced 38 grams of total plasma protein produced approximately 21 grams of albumin and 17 grams globulin. The addition of 100 grams bran flakes to a kidney basal diet results in the formation of about 12 grams albumin and 11 grams globulin. Since it has been shown that 100 grams casein will yield only 5 grams albumin and 7 grams globulin, and 100 grams gelatin 5 grams or less of each, it becomes apparent that, as measured by phasmapheresis, diet regulates globulin production equally as well as albumin."

The question remains, however: Does normal globulin originate only from nonessential amino-acids or does it require for its synthesis an adequate supply of several or even all of the *essential* amino-acids?

Chemical evidence concerning the structure of globulin should help to clarify the problem of its synthesis. Although complete amino-acid analyses of normal serum globulin are still lacking, there is evidence that it contains several of the amino-acids essential either for adequate growth of the white rat or for maintenance of nitrogen equilibrium in man. Improved methods of amino-acid analysis are necessary, however, before a final answer can be given. In the meantime we are utilizing a biologic method for the evaluation of protein quality which furnishes additional evidence that serum globulin probably contains all of the amino-acids essential for the growing rat.[15] The method consists in the daily ingestion by a hypoproteinemic adult white rat of known quantities of a test-protein, followed by the recording of weight recovery and serum protein regeneration at the end of a seven-day feeding period. The daily ration is adequate in calories, vitamins and salts, and has as its principal source of nitrogen the protein to be tested. An incomplete protein, such as gelatin, for example, will induce only slight serum protein regeneration and weight recovery, whereas a complete protein will cause both a marked serum protein regeneration and weight recovery. We have demonstrated by this method that purified globulins, of either bovine or human origin, and composed predominantly (over 90 per cent) of *gamma* globulin, are also high quality proteins. Thus in every instance in which they were tested, serum protein regeneration and weight recovery resulted practically as effectively as in animals fed the highest quality meat proteins.

II. THE RELATIONSHIP OF PROTEIN INGESTION TO ANTIBODY SYNTHESIS

All recent evidence has tended to strengthen the view that an antibody is but a specifically-modified molecule of normal globulin. An antibody molecule, therefore, in common with a normal globulin molecule, must be synthesized from amino-acids present in the food or stored and available in the protein reserves. But, inasmuch, as only a portion of the globulin fraction of normal serum contains antibody, more attention must be given to the antibody-containing portion. This so-called *gamma* fraction is characterized by its slow mobility in the Tiselius electrophoretic cell. Although it may not contain every type of antibody, at least several kinds have been demonstrated in immune serums from the horse, rabbit, rat, monkey and man. These antibodies, moreover, are interchangeable between species. Thus, rabbit and horse immune serums are suitable for antibacterial therapy in man, and human serum can react specifically with micro-organisms which infect lower animals. Furthermore, nutritional conditions which influence the fabrication of blood proteins, as for example, albumin, globulin and hemoglobin, act essentially alike in the dog, rat and man. There is reason to believe, therefore, that these basic mechanisms of protein synthesis function similarly in all mammalian species. Furthermore, the concentration of the globulin fraction in man tends to rise in infections characterized by hyperproteinemia, such as tuberculosis, syphilis, lymphogranuloma, sarcoid, leichmaniasis,

rheumatoid arthritis, lupus erythematosus, *etc.* In some instances, also, there is a concomitant increase in the amount of *gamma* globulin.

Normal human serum contains around 25 mg. per Ml. of globulin; of this, approximately 8 mg., or about one-third, may be *gamma* globulin.[16] It is in this one-third of the globulin fraction, therefore, that the antibodies are found. Furthermore, inasmuch as the rise and fall in concentration of antibody globulin may parallel that of total globulin, a hypoglobulinemia may be accompanied by a corresponding decline in the amount of *gamma* globulin. Conversely, however, hyperglobulinemia does not necessitate an increase in the *gamma* fraction or in its antibody-containing portion, because during the time of the rise, as for example, during the process of reversal of the albumin-globulin ratio, antigenic stimulation might be absent. In hyperimmune animals, however, as much as 35 per cent of the total globulin fraction has been found to be antibody globulin. It should be borne in mind, also, that inasmuch as more than two-thirds of the total globulin fraction presumably has no relationship to antibody content, concentration of this fraction in the blood (*alpha* and *beta* globulin) might rise and yet have no relationship whatever to a decreased content of antibody *gamma* globulin or to depletion of the *gamma* globulin reserves.

III. THE RELATIONSHIP OF PROTEIN DEFICIENCY TO ANTIBODY SYNTHESIS AND TO ACQUIRED RESISTANCE TO INFECTION

If our interpretation of the foregoing facts is correct, protein inadequacy and concomitant depletion of the protein reserves should lead in time to impairment of the capacity of a protein-depleted animal or patient to fabricate *gamma* globulin. This we have shown to be true for the production of agglutinins, precipitins and hemolysins, both in rabbits and white rats. Such animals also react less effectively against certain spontaneous and induced infections.

Our experiments with rabbits indicated that severe protein deficiency, in association with general undernutrition, lowered markedly the levels of serum protein and hemoglobin concentration and induced a significant decrease both in the rate and quantity of antibody output. In order to ascertain more precisely the effects of protein depletion upon the antibody-forming mechanism, experiments utilizing the hypoproteinemic adult white rat were performed in animals, which, during the period of protein depletion, were fed a diet presumably adequate in other nutritional elements. When the rats had become markedly hypoproteinemic they were injected intravenously with antigen in order to determine the output of antibody. As controls, rats which during the period of protein depletion in the first group, had been fed a diet identical in every respect except for the presence of 22 per cent casein, were similarly injected.

The compositions of the rations fed to the two groups of animals were as follows:

(Per 100 Gm. of Ration)

	Ration 3E‡	Ration 3C‡
Ground fresh carrots	30 Gm.	30 Gm.
Ruffex	5 Gm.	5 Gm.
Lard	4 Gm.	4 Gm.
Cornstarch	44 Gm.	22 Gm.
Casein (vit. test Smaco)	none	22 Gm.
Osborne & Mendel salt mixture*	4 Gm.	4 Gm.
Dried Brewer's yeast (Mead Johnson)	2 Gm.	2 Gm.
Liver concentrate (Wilson & Co. 20:1)†	1 Gm.	1 Gm.
Water	10 Ml.	10 Ml.
Calcium pantothenate	200 *gamma*	200 *gamma*
Pyridoxine HCl	200 *gamma*	200 *gamma*
Riboflavin	500 *gamma*	500 *gamma*
Choline chloride	100 mg.	100 mg.
Oleum percomorphum:		
—vitamin A	200 USP units	200 USP units
—vitamin D	29 USP units	29 USP units

* Hawk and Osers modification plus 1 Gm. each of copper sulphate and zinc chloride added to the trace elements.

†Generously supplied by the pharmaceutical laboratories of Wilson and Company, Chicago.

‡Ration 3E contained approximately 2 per cent of protein in contrast to the 22 per cent of case in ration 3C. Both rations contained two rich sources of B complex vitamins in addition to those present in the raw carrots and added as synthetic vitamins.

The rats (Sprague-Dawley strain) were kept in large wire-mesh cages with wire bottoms, six animals per cage; throughout the experiment each rat received 20 grams of ration per day. Consumption was good in both groups, falling off only in the animals on the low-protein group as they lost weight. Water was given *ad libitum* together with 20 grams of leaf lettuce per rat per week.

Table I summarizes the essential data with respect to initial weights, length of dietary periods, weight gains or losses, and concentrations of serum protein and hemoglobin at the time of injection of the antigen.

TABLE I

					Av. Values at Time of Antigen Injection		
Exp. No.	No. of Animals	Av. Initial Wts. (Gm.)	Diets	Diet Period	Wt. Loss or Gain (Gm.)	Serum Protein Gm. % Concentration	Hb. Gm. %
1	12	304	3C	5 mos.	+ 23	6.68	15.1
	12	316	3E	5 mos.	—119	4.24	10.2
2	12	307	3C	6 mos.	+ 49	6.71	14.3
	12	315	3E	6 mos.	—151	4.39	7.5

Blood for the various determinations was obtained from a tail vein in 1 Ml. amounts. Serum protein concentrations were determined routinely by the specific gravity method of Barbour and Hamilton.[17] This method, in our experience, has checked consistently (± 0.2) with the micro-Kjeldahl method of Ma and Zuazaga[18] and the direct biuret method of Kingsley.[19] It affords a relatively simple and rapid way of determining serum protein concentrations in small quantities of serum or plasma. Hemoglobin concentrations were determined by means of the Dick-Stevens photo-electric hemoglobinometer.[20]

After the above data had been obtained all rats were injected simultaneously in a tail vein with 1 Ml. of a 0.25 per cent suspension of washed sheep's erythrocytes. The cells were always centrifuged at the same speed and time at the last washing before preparation of the suspension either for injection of hemolysin titration. A sample of blood serum taken from each animal before the antigen was injected was titrated for evidence of naturally-occurring hemolysin, starting at a serum dilution of 1–60; only an occasional serum contained hemolysin and none showed complete hemolysis at this dilution of serum.

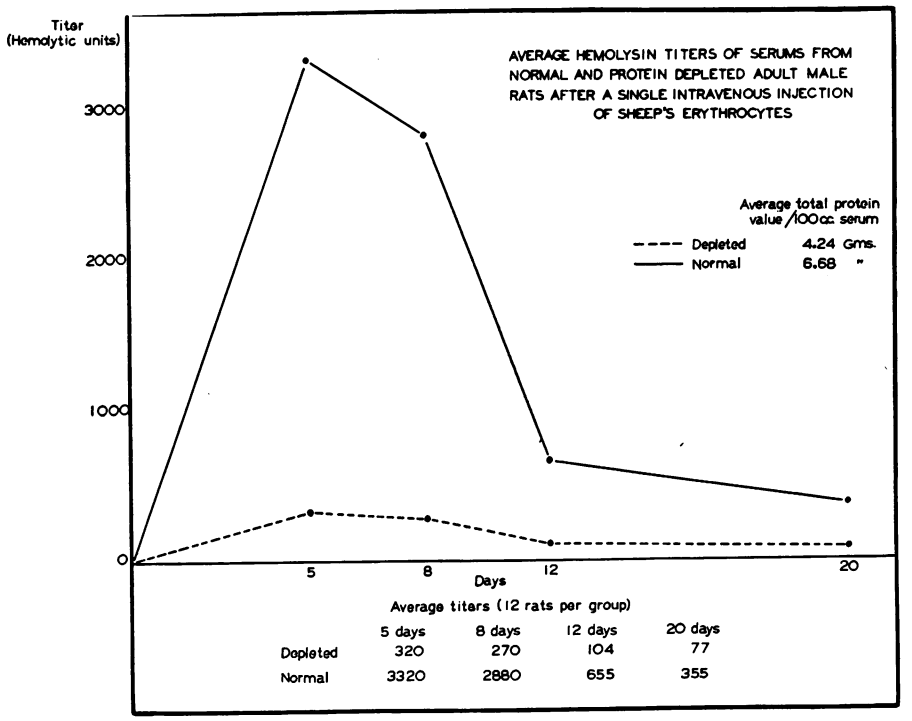

CHART I

For the hemolysin titrations a method used originally by Hektoen[21] was employed, modified in that a 2 per cent suspension of washed sheep's erythrocytes in 0.85 per cent salt solution (0.2 Ml.) was mixed with 0.3 Ml. of appropriately diluted inactivated immune serum and from 0.1 to 0.15 Ml. of a 1–6 dilution of pooled fresh guinea-pig serum. The complement in the guinea-pig serum was always titrated in association with the pooled inactivated serums from three protein-deficient rats which had been injected intravenously with sheep's erythrocytes; from one and one-half to two units were used in the final titrations; the titrations to determine hemolytic activity were all done simultaneously, using the double dilution method at a beginning serum dilution of 1–60. The hemolytic titer was taken as the highest dilution of serum showing complete hemolysis, all titers being recorded after incuba-

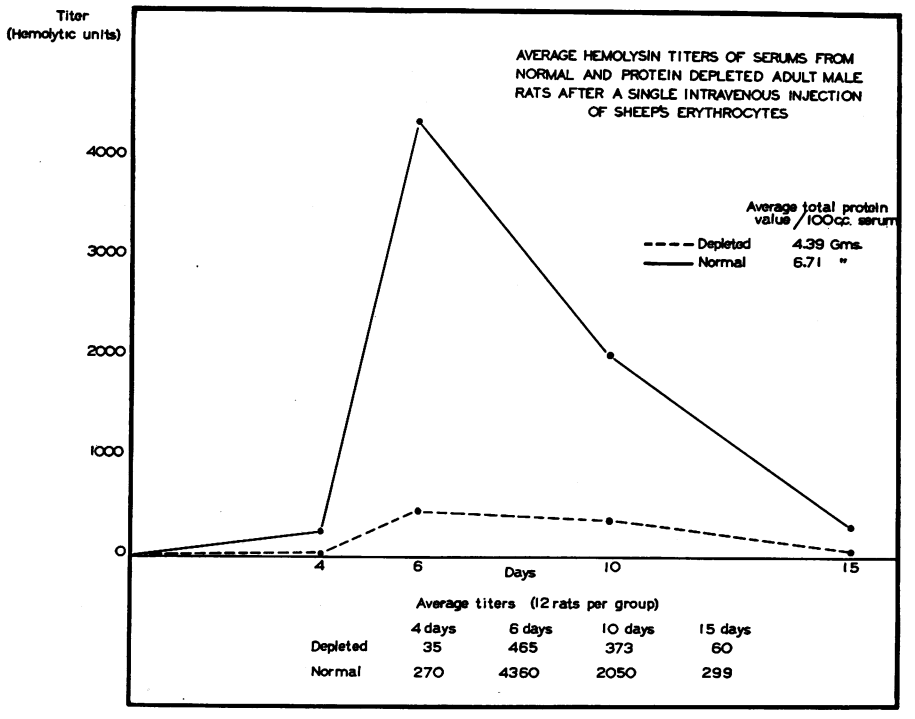

CHART II

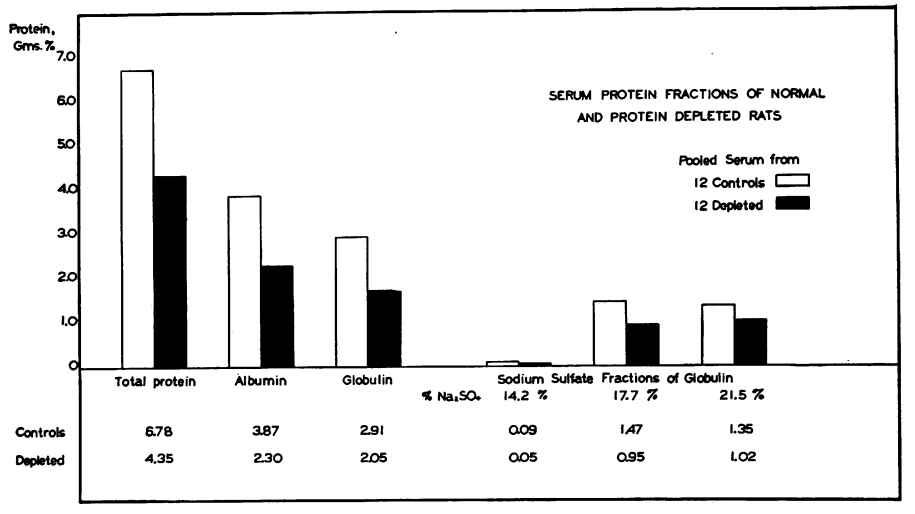

CHART III

tion at 37° C. in a water bath for two hours and storage overnight in a refrigerator at approximately 4° C.

The results for two groups of animals are shown graphically in Charts 1 and 2, where it is seen that at the peak of antibody-output (6–8 days) the average hemolysin titers for the normal sera were approximately ten times higher than those from the protein-depleted rats; the differences are also striking at the later periods. In other words, the animals with abundant protein reserves and an adequate protein intake were able, on the average to fabricate approximately ten times as much antibody as were those with depleted reserves and the low-protein diet continued during the period of antibody production.

Besides demonstrating a decreased capacity to fabricate antibody, the protein-depleted rats also manifested a definitely increased tendency to develop

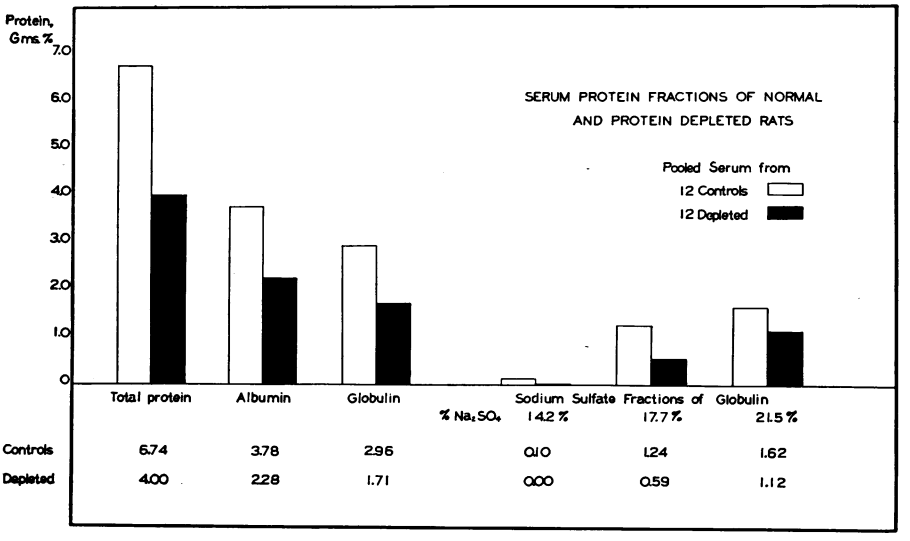

CHART IV

spontaneous infection. For example, in the first experiment three of the twelve hypoproteinemic rats died during the following three weeks; at the end of this period, moreover, four others exhibited varying degrees of chronic abscessive pneumonia. In the second experiment nine of the twelve hypoproteinemic rats showed, when sacrificed, a chronic abscessive pneumonia. In contrast, none of the control rats in either group showed any gross evidence of pulmonary infection.

In addition to testing antibody titers the serums were also pooled in order to ascertain the comparative concentrations of albumin and the various globulin fractions in each group. The results are shown graphically in Charts 3 and 4.

Procedures.—Serums from 12 rats of each group were pooled (0.1 Ml. per

rat) and a total protein determination made on 0.1 Ml. in duplicate (biuret method).[19] The remaining portions of serum were then mixed with various concentrations of sodium sulfate according to the procedure of Howe.[22, 23] Serum (0.3 Ml.) was added to 4.5 Ml. of a solution of sodium sulfate to give a final sulfate concentration of 14.2 per cent, 17.7 per cent and 21.5 per cent. After mixing, the tubes were allowed to stand overnight at room temperature (around 25° C.). Next day the mixtures were centrifuged and residual protein determined in the supernates. From the results shown in Charts 3 and 4, it is evident that the rats on the low protein diet developed both hypoalbuminemia and hypoglobulinemia, with diminished concentration of all three globulin fractions, *viz.*, euglobulin, and pseudoglobulins I and II.

Discussion.—We have demonstrated by both immunologic and chemical methods that in the blood serums of protein-deficient rats there is a lowered concentration of antibody globulin and of three globulin fractions. This indicates definitely the adverse action of protein deficiency upon protein reserves and their capacity to generate serum proteins. In many surgical conditions there is also both a preoperative and a postoperative loss of various serum protein fractions, as into exudates, from hemorrhage or through the kidneys. Furthermore, undernutrition caused by an inadequate intake, absorption or utilization of high-quality protein may also lead to a steady depletion of the tissue protein reserves, both before and after operation. This loss of tissue proteins may finally become critical if and when a potentially pathogenic micro-organism enters tissues unprepared to mobilize the forces of acquired resistance.

It is obvious, moreover, from these experiments, that reliance upon the so-called albumin-globulin ratio for the determination of the state of the tissue protein reserves is inadequate. We need to know not only whether the globulins are in a lowered concentration, but particularly whether there is a diminished concentration of the *gamma* globulin fraction. If the total serum globulin concentration is low, the *gamma* fraction will also probably be low; if, on the other hand, the serum globulin is present in normal or elevated concentration, either condition may be due to an increased concentration of *alpha* or *beta* globulin and reveal nothing about the concentration of *gamma* globulin. What is actually needed is a quantitative clinical method for determining the concentration of *gamma* globulin.

In the absence of such a method dependence must be placed upon clinical procedures, such as evaluation of the extent of the preoperative weight loss and total serum protein determinations. The latter must be done, however, after proper attention to the problem of rehydration of the patient's tissues. If the total serum protein concentration is found to be less than 5 grams per 100 Ml. of serum, preoperative protein repletion should be attempted, if possible, either by ingestion or intravenous administration of high quality proteins, in order both to reduce the tissue protein deficit and to build up a backlog of protein reserves available for antibody production in the

event of a developing infection. For unless the antibody globulin reserves can be repleted through the utilization of a proper supply of essential amino-acids, infection cannot be effectively combated through the intermediation of the antibody mechanism.

Since *gamma* globulin is a high quality protein, it presumably contains many if not most of the essential amino-acids. For its synthesis, therefore, it is necessary to provide an abundance of essential amino-acids in the protein reserves or in the daily food. In experiments to be reported later we have demonstrated that, when high quality proteins are fed to hypoglobulinemic rats, the animals quickly regenerate immune globulin, as evidenced by the rapid recovery of their capacity to produce specific antibody. This does not happen, however, when poor-quality proteins are fed. The inference seems warranted, therefore, that in patients with depleted globulin reserves, repletion necessitates the ingestion or intravenous administration of proteins containing all of the essential amino-acids. The further effects will obviously depend upon the patient's ability to convert these amino-acids into tissue proteins, including antibodies.

SUMMARY AND CONCLUSIONS

Both the frequency and severity of postoperative infection depend largely upon the capacity of the individual to mobilize the protective forces of natural and acquired resistance. These forces are dependent, basically, upon protein metabolism, acting through the agency of amino-acids ingested in the food or readily available in the tissue protein reserves. Attention has been directed in the present discussion to the rôle of the blood and tissue globulins, especially the *gamma* globulin fraction, in the mechanism of acquired resistance, and to their origin from dietary amino-acids. Evidence has been presented indicating that, to the extent that protein deficiency leads to depression of the capacity of certain tissues to fabricate antibody globulin, the potential ability to elaborate specific antibodies is concomitantly impaired. The implications of such protein depletion with respect to starvation, particularly in surgical patients, are discussed.

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